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PATENT- OG VAREMÆRKESTYRELSEN

USE OF EHDP FOR THE TREATMENT OF CALCIUM PYROPHOSPHATE DEPOSTION DISEASE

FIELD OF THE INVENTION

The present invention relates to the use of alkyl-1,1-bisphosphonic acid derivatives for the preparation of a medicament for the treatment of calcium pyrophosphate deposition disease (CPDD) primarily pseudogout and chondrocalcinosis, in a mammal More specifically, the present invention relates to the use of alkyl-1,1-bisphosphonic acid derivatives, said derivatives being especially adapted to be administered to subjects suffering from CPDD

BACKGROUND OF THE INVENTION

The term "calcium pyrophosphate deposition disease" (CPDD) is used to describe the clinical syndrome of acute gout-like arthritis associated with the presence of calcium pyrophosphate crystals in the synovial fluid of a mammal Other terms used to define the clinical syndrome of acute gout-like arthritis are "Calcium pyrophosphate dihydrate crystal deposition disease" (CPPD Disease), "pyrophosphate gout" or "pseudogout"

The terms "Pseudogout" or "CPDD" are now and then compared with true "urate gout", (Ryan, L M et al , "Calcium pyrophosphate crystal deposition disease, pseudogout and articular chondrocalcinosis", In Arthritis and Allied Conditions, A Textbook in Rheumatology, 13th edition, Vol, II, Ed W J Kooperman, 1997, pp 2103-2125) Patients suffering from symptomatic arthritis, such as "urate gout", however do not experience acute attacks as do patients suffering from CPDD (e g acute inflammation and swelling of joints) Hence, it is vital that the terms "Pseudogout" or "CPDD" are reserved to the acute episodes associated with the precipitation of calcium pyrophosphate crystals in e g the synovial fluid

CPDD is seldom seen in patients below age 50, the overall incidence of this disease occurs in the later years of life The most common association of calcium pyrophosphate crystal deposition is the formation of these crystals in the joints of aged patients (Kenneth P H Pritzker, "Crystal associated Arthropathies What's New on Old Joints", J American Geriatrics Society, 28 (1980) 439-445)

The calcium pyrophosphate crystal deposits are topologically confined to the hyaline cartilage, the fibrocartilage in the meniscus of the knee, the annulus fibrosus of the intervertebral disc, the synovial fluid, or the synovium and tendon insertions (Kenneth P H Pritzker et al , "Crystal associated Arthropathies What's New on Old Joints", J American Geriatrics Society, 28 (1980) 439-445)

The calcium pyrophosphate crystal deposits are also frequently known to form or precipitate in the articular cartilage, particularly in elderly people (Ryan, L M et al , "Calcium pyrophosphate crystal deposition disease, pseudogout and articular

chondrocalcinosis", In Arthritis and Allied Conditions, A Textbook in Rheumatology, 13th edition, Vol, II, Ed W J Kooperman, 1997, pp 2103-2125)

The calcium pyrophosphate crystal deposits are known to especially form in the synovial fluid, particularly in elderly people

The formation of calcium pyrophosphate crystal deposits are believed to be caused by an increase in the concentration of pyrophosphate, PP, caused by the changes in the PP metabolism of chondrocytes (Ryan et al , "Understanding inorganic pyrophosphate metabolism toward prevention of calcium pyrophosphate dihydrate crystal deposition, Annals of the Rheumatic Diseases 54 (1995) 939)

The disodium salt of ethane-1-hydroxy-1,1-bisphosphonic acid, abbreviated EHDP, and a series of similar bisphosphonates are used in the treatment of osteoporosis and for the prevention of bone fractures in connection to various cancer diseases Such compounds are known under various trade names, e g "Didronel" or "Aredia" or "Fosamax"

EP 0924293 discloses a fabric care composition including the compound hydroxy-ethane-1,1-diphosphonic acid (HEDP) and its use in order to inhibit the formation of inorganic microcrystals

US 3 683 080 discloses a composition which may include effective amounts of polyphosphonates, such as ethane-1-hydroxy-1,1-bisphosphonic acid (EHDP), for the inhibition of anomalous deposition and mobilisation of calcium phosphates in animal tissue

EP 563096 discloses anti-inflammatory compositions comprising salicylic acid-, phenylacetic acid-, anthranilic acid- based inflammation inhibitors and an amount of an organic phosphonic acid or one of its salts or esters, said compositions being suitable for treating rheumatoid arthritis, bone infections and bone degradation

US 4 812 304 discloses a method for treating or preventing osteoporosis in humans, the method amongst others comprising a bone resorption period during which ethane-1-hydroxy-1,1-diphosphonic acid (EHDP) is administered

US 5 882 656 discloses pharmaceutical compositions of bisphosphonic acids for the treatment of disturbances involving the calcium or phosphate metabolism Suitable organophosphonate compounds include 4-amino-1-hydroxybutylidene-1,1-bisphosphonic acid

US 6 221 861 discloses a method for the treatment of an animal with pyrophosphate gout comprising administering an effective amount of calcium antagonists, such as phenylalkylamines, dihydropyridines or benzothiazepines

JP 10017493 discloses an external anti-inflammatory or antiallenc skin composition containing at least one calcium ion blocking agent consisting of hydroxyethanediphosphonic acid (EHDP)

BRIEF DESCRIPTION OF THE INVENTION

In spite of the substantial body of literature relating to the component of interest within the present invention, such as ethane-1-hydroxy-1,1-bisphosphonic acid (EHDP), the use of said compound and its acid derivatives for the treatment of Calcium Pyrophosphate Deposition Disease (CPDD) or pseudogout do not appear to have been appreciated heretofore

According to the present invention, bisphosphonate acid derivatives, such as alkyl-1,1-bisphosphonic acids (EHDP), have the ability to severely inhibit the rate of growth of various forms of calcium pyrophosphate crystals when present in medically relevant concentrations A particular embodiment of the present invention, as will be shown in the following, is the ability to severely retard the spontaneous precipitation of various forms of calcium pyrophosphate crystals from solutions supersaturated with respect to calcium pyrophosphate Rates of dissolution are however unchanged

According to the present invention, EHDP can be used in yet another context, namely for the manufacture of a medicament for the prevention or treatment of secondary caries, i e caries that forms at the interface between the natural dental material (enamel, dentine, cementum and root material) and the filling material

Experiments indicate that the use of EHDP in this context is able to inhibit or reduce the development of secondary caries in an animal, preferably a human It is believed that EHDP acts as a chelating agent and thereby reduces or inhibits the precipitation of calcium pyrophosphates beneath of or in the vicinity of the "primary" caries Hence, use of EHDP prior to any medical operation or procedure on a tooth subject to "primary caries" will have the advantage of providing a stable tooth in which substantially no secondary caries will be susceptible to form

It is anticipated that, when EHDP is applied to a tooth subject to "primary caries", it will result in, amongst others, an unchanged adhesion between the natural dental material (enamel, dentine, cementum and root material) and the filling material, as compared with a similar tooth that has not been subjected accordingly with EHDP

DETAILED DESCRIPTION OF THE INVENTION

CPDD

The present invention provides use of an effective amount of an alkyl-1,1-bisphosphonic acid derivative, for the preparation of a medicament for the treatment of calcium pyrophosphate deposition disease (CPDD) in an animal The animal is preferentially a human As used herein, CPDD includes pseudogout, chondrocalcinosis, and any other disease caused by the deposition of calcium pyrophosphate crystals in the body

The organophosphonate compounds critical to the practice of the present invention are discussed more fully hereinafter

Organophosphonate compounds useful herein are of the formula

$$R^1\text{-}C(PO_3H_2)_2\text{-}R^2 \qquad (I)$$

wherein R^1 is selected from hydrogen and C_{1-6}-alkyl, and R^2 is selected from hydroxy, amino, $-CH_2COOH$, $-CH_2PO_3H_2$ and - $CH_2CH_2PO_3H_2$

In a preferred embodiment of the present invention, R^1 is selected from C_{1-6}-alkyl and R^2 is selected from hydroxy

In a further preferred embodiment of the present invention, R^1 is selected methyl and R^2 is selected from hydroxy

Among the organophosphonates encompassed by formula (I) are methane-hydroxybisphosphonic acid and ethane-1-amino-1,1-bisphosphonic acid An even more preferred organophosphonate according to the present invention is ethane-1-hydroxy-1,1-bisphosphonic acid, with the formula $CH_3C(OH)(PO_3H_2)_2$ (abbreviated EHDP) Although any pharmaceutically acceptable salt of ethane-1-hydrox-1,1-bisphosphonic acid can be used in the practice of the present invention, the trisodium hydrogen salt, the disodium hydrogen salt, the monosodium hydrogen salt, and the mixtures thereof are preferred, e g

$$[CH_3C(OH)(PO_3)_2H]^{3-}, 3Na^+ \qquad (II)$$

Other pharmaceutically acceptable salts are, e g , those described in Remington's - The Science and Practice of Pharmacy, 20th Ed Alfonso R Gennaro (Ed), Lippincott, Williams & Wilkins, ISBN 0683306472, 2000, and in Encyclopaedia of Pharmaceutical Technology

The medicament may be formulated according to conventional pharmaceutical practice, see, e g , Remington's - The Science and Practice of Pharmacy, 20th Ed Alfonso R Gennaro (Ed), Lippincott, Williams & Wilkins, ISBN 0683306472, 2000, and in Encyclopaedia of Pharmaceutical Technology Typically, the compounds defined herein are formulated with (at least) a pharmaceutically acceptable carrier or excipient Pharmaceutically acceptable carriers or excipients are those known by the person skilled in the art

As used herein, the term "pharmaceutically acceptable carrier" denotes a solid or a liquid filler or an encapsulating substance Such substances may be selected from the group consisting of sugars such as lactose, glucose and sucrose, starches such as corn starch and potato starch, cellulose and its derivatives, such as sodium carboxymethylcellulose, ethylcellulose, cellulose acetate, gelatine, talc, malt, stearic acid, vegetable oils, polyols such as propylene glycol, polyethylene glycol, agar as well as other non-toxic compatible substances used in pharmaceutical compositions

The administration route of the compounds as defined herein may be any suitable route which leads to a concentration in the blood or tissue corresponding to a therapeutic concentration Thus, e g , the following administration routes may be applicable although the invention is not limited thereto the oral route, the parenteral route, the cutaneous route or the nasal route It should be clear to a person skilled in the art that the choice of administration route depends on the physico-chemical properties of the compound together with the age and weight of the patient and on the particular the condition and the severity of the same

The alkyl-1,1-bisphosphonic acid derivatives as defined herein may be contained in any appropriate amount in a pharmaceutical composition, the pharmaceutical composition comprising an amount of about 1-95% by weight of the total weight of the composition The composition may be presented in a dosage form which is suitable for the oral route, the parenteral route, the cutaneous route or the nasal route Thus, the composition may be in form of, e g , tablets, capsules, pills, powders, granulates, suspensions, emulsions, solutions, gels including hydrogels, pastes, creams, plasters, drenches, delivery devices, injectables, implants, sprays, aerosols and in other suitable form

According to the present invention, a medical relevant concentration of the alkyl-1,1-bisphosphonic acid derivative is 5 to 35 µM (5 to 35 micro Molar) when dissolved in the animal body Other medical relevant concentrations may however be used, depending on the individual condition of the subject, i e the severity and course of the disease, the subjects health and response to the particular treatment Hence, other medical relevant concentrations of the alkyl-1,1-bisphosphonic acid derivative may be lower, such as 0 5-4 µM, or higher, such as 36 - 50 µM

In one aspect of the present invention, the CPDD is confined to the hyaline cartilage, the fibrocartilage in the meniscus of the knee, the annulus fibrosus of the intervertebral disc, or the synovium and tendon insertions

In another aspect of the present invention, the CPDD is especially confined to the synovial fluid or to the articular cartilage of the mammal, the mammal of which preferably is a human

Secondary Caries

In another preferred aspect of the present invention, an alkyl-1,1-bisphosphonic acid derivative is used for the manufacture of a medicament for the prevention or treatment of secondary caries in an animal, preferentially a human

The term "Secondary Caries", when used herein, is defined as caries that forms beneath or behind "primary caries", i e beneath or behind caries that has already formed and subsequently been treated according to any conventional means known to the person skilled in the art

The secondary caries may be confined to the interface of the natural dental material (enamel, dentine, cementum and root material) and the filling material The filling material is typically selected from the group consisting of amalgam and plastic

The alkyl-1,1-bisphosphonic acid derivative used for the manufacture of a medicament for the prevention or treatment of secondary caries is selected from the compound of the formula II

$$R^1\text{-}C(PO_3H_2)_2\text{-}R^2 \qquad \text{(II)}$$

wherein R^1 is selected from hydrogen and $C_{1\text{-}6}$-alkyl, and R^2 is selected from hydroxy, amino, $-CH_2COOH$, $-CH_2PO_3H_2$ and - $CH_2CH_2PO_3H_2$

According to an aspect of the present invention, R^1 is preferentially $C_{1\text{-}6}$-alkyl and R^2 is hydroxy

According to another aspect of the present invention, R^1 is preferentially methyl and R^2 is hydroxy

In a preferred embodiment of the present invention, the bisphosphonic acid derivatives are selected from ethane-1-hydroxy-1,1-bisphosphonic acid, methanehydroxybisphosphonic acid or ethane-1-amino-1,1-bisphosphonic acid

In the context of "secondary caries", it is anticipated that EHDP is to be applied onto a tooth subject to "primary caries" It is anticipated that the application of said compound is able to prevent or inhibit the subsequent formation of "secondary caries", i e the formation of caries below, behind or in the vicinity of the "primary caries" Hence, the skilled person will see that the application of EHDP to a tooth subject to primary caries, i e the application of EHDP prior to filling the primary caries with a filling material, is able to inhibit the precipitation or growth of calcium pyrophosphates (originating from the enamel and/or dentine), and hence minimise the tendency to develop "secondary caries"

EXAMPLES

The following examples are presented to illustrate the present invention and to assist one of ordinary skill in making and using the same It will be appreciated that the examples are not intended in any way to otherwise limit the scope of the invention

Stock crystals of m-CPPD, for use in the following examples, were prepared according to the method of Mandel et al (Calcium pyrophosphate crystal deposition disease preparation and characterisation of crystals, J Crystal Growth 87 (1988) 453-462)

X-ray diffraction patterns showed that the prepared stock was mainly m-CPPD but with some t-CPPD, the most stable form of calcium pyrophosphate (CPP) The specific surface area of the m-CPPD stock is 1 35 m^2/g The prepared crystals have a short needle-like shape with the longest dimension measuring about 10 µm

Stock crystals of columnar t-CPPD, for use in the following examples, were prepared according to the method of Christoffersen et al (Kinetics of dissolution of triclinic calcium pyrophosphate dihydrate crystals, J Crystal Growth, 203 (1999) 234-243) The specific surface area of the columnar t-CPPD stock crystals is 0 8 m^2/g

Stock crystals of acicular t-CPPD, for use in the following examples, were prepared according to the method of Christoffersen et al (Kinetics and mechanism of dissolution and growth of acicular triclinic calcium pyrophosphate dihydrate), to be published The specific surface area of these stock crystals is 2 8 m^2/g

Stock crystals of m-CPPTβ, for use in the following examples, were prepared according to the method of Christoffersen et al (Growth and precipitation of a monoclinic calcium pyrophosphate tetrahydrate indicating auto-inhibition at pH 7, J Crystal Growth, 212, 500-506) The specific surface area of the m-CPPTβ stock crystals is 6 m^2/g

The compound EHDP, for use in the following examples, was 1) provided by Procter and Gamble in the form of a disodium salt, and 2) provided by Tokyo Kasei Kogyo Co , Japan, in the form of the tetra-acid

Example I

The following examples serve to illustrate the effect that the presence of EHDP has on solutions that 1) are supersaturated (or unsaturated) with calcium pyrophosphate and 2) solutions that further comprise various stock crystals of calcium pyrophosphate dihydrate The various stock crystals used in the following examples are monoclinic CPPD (m-CPPD), triclinic CPPD (t-CPPD), columnar t-CPPD, acicular t-CPPD and monoclinic CPPT (m-CPPTβ)

When calcium pyrophosphate is deposited (growth or precipitation), the solution becomes more acidic Hence, in order to maintain a constant pH-value, a base must be added (KOH used herein) The amount of KOH added to solutions supersaturated with calcium pyrophosphate is therefore a measure of the extent and rate of the deposition of the calcium pyrophosphate crystals

When calcium pyrophosphate is dissolved, the solution becomes more basic Hence, in order to maintain a constant pH-value, an acid must be added (HNO_3 used herein) The amount of acid added to solutions supersaturated with calcium pyrophosphate is therefore a measure of the extent and rate of the dissolution of the calcium pyrophosphate crystals

The supersaturation, S, is defined as

$$S = \frac{a}{a_S} = \left(\frac{IP}{K_S}\right)^{\frac{1}{3}}$$

where a is the mean ion activity, IP the activity product and K_S the solubility product The index s refers to a saturated solution In the following examples, the supersaturation S is calculated using an ion speciation programme and "Kielland activity coefficients", the calculation of which is known by the person skilled in the art

Table 1 below serves to illustrate the growth of columnar t-CPPD, when 10 mg/L columnar t-CPPD is added to a solution supersaturated with an initial calcium pyrophosphate concentration of 0 15 mM, i e $C_{Ca,0} = 2C_{PP,0} = 0$ 15 mM During the experiment, pH is kept constant at 6 5 by titration with 2 0 mM KOH The initial supersaturation, S_0, is 6 3

Experiment number	C_{EHDP}/µM	Time / h	V_{KOH}/mL	C_{Ca} / mM
1	0	3	4 3	0 13
2	1	3	2 1	0 14
3	5	3	0	0 15

Table 1 Growth of columnar t-CPPD, pH = 6 5, temperature is 37 0 ± 0 1 °C

From table 1 it is seen that the growth of columnar t-CPPD over 3 hours is reduced by 50% if the concentration of EHDP (C_{EHDP}) is 1 µM, i e from 4 3 mL KOH to 2 1 mL KOH When the concentration of EHDP (C_{EHDP}) is 5 µM, the rate of growth or precipitation of columnar t-CPPD is totally blocked, i e the pH is kept constant without the addition of KOH

Table 2 below serves to illustrate the growth of acicular t-CPPD, when 10 mg/L acicular t-CPPD is added to a solution supersaturated with an initial calcium pyrophosphate concentration of 0 15 mM, i e $C_{Ca,0} = 2C_{PP,0} = 0$ 15 mM During the experiment, pH is kept constant at 6 5 by titration with 2 0 mM KOH The initial supersaturation, S_0, is 6 3

Experiment number	C_{EHDP}/µM	Time / h	V_{KOH}/mL	C_{Ca} / mM
4	0	3 5	5 4	0 12
5	1	4	3 4	0 13
6	10	4	0 1	0 14

Table 2 Growth of acicular t-CPPD, pH = 6 5, temperature is 37 0 ± 0 1 °C

From table 2 it is seen that the growth of acicular t-CPPD is severely inhibited when the concentration of EHDP (C_{EHDP}) is 1 µM, i e from 5 4 mL KOH to 3 4 mL KOH, (corresponding to a reduction in the growth of acicular t-CPPD of roughly 40 %) When the concentration of EHDP (C_{EHDP}) is 10 µM, the rate of growth or precipitation of acicular t-CPPD is substantially blocked, i e the pH is kept constant by the addition of 0 1 mL KOH

Table 3 below serves to illustrate the deposition of calcium pyrophosphate (growth of m-CPPD and/or spontaneous precipitation of calcium pyrophosphate, CPP), when a solution comprising 10 mg/L m-CPPD is added to a solution supersaturated with an initial calcium pyrophosphate concentration of 0 17 mM, i e $C_{Ca,0} = 2C_{PP,0} = 0\ 17$ mM During the experiment, pH is kept constant at 7 0 by titration with 2 0 mM KOH The initial supersaturation, S_0, is 5 6

Experiment number	C_{EHDP} / µM	Time / h	V_{KOH} / mL	C_{Ca} / mM
7	0	3	13 0	0 09
8	1	3	5 3	0 12
9	10	3	0	0 14

Table 3 Growth of m-CPPD, pH = 6 5, temperature is 37 0 ± 0 1 °C

From table 3 it is seen that the growth and/or precipitation of m-CPPD over 3 hours is reduced by approximately 50% if the concentration of EHDP (C_{EHDP}) is 1 µM, i e from 13 0 mL KOH to 5 3 mL KOH When the concentration of EHDP (C_{EHDP}) is 10 µM, the rate of growth or precipitation of m-CPPD is totally blocked, i e the pH is kept constant without the addition of KOH Spontaneous precipitation of calcium pyrophosphate is observed without the addition of EHDP

Table 4 below serves to illustrate the growth m-CPPTβ, when 10 mg/L m-CPPTβ is added to a solution supersaturated with an initial calcium pyrophosphate concentration of 0 125 mM, i e $C_{Ca,0} = 2\ C_{PP\ 0} = 0\ 125$ mM During the experiment, pH is kept constant at 7 0 by titration with 2 0 mM KOH The initial supersaturation, S_0, is 3 1

Experiment number	C_{EHDP}/µM	Time / h	V_{KOH} / mL	C_{Ca} / mM
10	0	4	11 5	0 053
11	1	4	10	0 062
12	5	4	0	0 11
13	10	4	0 6	0 12
14	10*	4	0 1	0 13
No crystals added				
15	0	4	9	0 064
16	10	4	0	0 13

Table 4 Growth of m-CPPTβ, when 10 mg/L m-CPPTβ is added to a solution supersaturated with calcium pyrophosphate, $C_{Ca,0} = 2C_{PP,0} = 0\ 125$ mM, pH kept constant at 7 0 by titration with 2 0 mM KOH, temperature is 37 0 ± 0 1 °C *EHDP from Kasei Kogyo Co , Japan is used

From table 4 it is seen that, for solutions supersaturated with calcium pyrophosphate, and with m-CPPTβ crystals added, the addition of 5 – 10 µM EHDP is able to adequately inhibit the deposition of calcium pyrophosphate, CPP, (growth of m-CPPTβ and/or the spontaneous precipitation of CPP) For example, the amount of KOH added in experiment number 10 (C_{EHDP} = 0 µM), is 11 5 ml, whereas the amount of KOH added in experiment number 12 (C_{EHDP} = 5 µM), is 0 ml, i e , the growth of m-CPPTβ and/or the spontaneous precipitation of CPP, is severely inhibited by the addition of 5 µM EHDP

Experiment number 15 and 16 serve to illustrate the effect when no crystals are added to a solution supersaturated with respect to calcium pyrophosphate, i e $C_{Ca,0} = 2C_{PP\,0} = 0\ 125$ mM It is seen that the addition of 10 µM EHDP is able to inhibit fully the spontaneous precipitation of calcium pyrophosphate, i e the amount of KOH added is 0 mL

Example II

Tables 5 and 6 below serve to illustrate the spontaneous precipitation of calcium pyrophosphate, CPP, at room temperature, with and without the addition of 10 µM EHDP The results from table 5 are obtained at a pH-value of approximately 7, whereas the corresponding results in table 6 are obtained at somewhat higher pH values, i e pH ranging from 7 0 - 7 8

The initial calcium concentration, $C_{Ca,0}$, is 0 9 mM in all the experiments S_0 is the supersaturation, as defined herein, at pH_p with respect to m-CPPTβ C_{PP} is the calcium pyrophosphate concentration, and C_{EHDP} is the concentration of the compound ethane-1-hydroxy-1,1-bisphosphonic acid t_p is the time, after mixing EHDP and m-CPPTβ, at which the onset of precipitation is observed, i e as a clear decrease in the pH-value When used herein, a "clear decrease" is defined as a decrease in pH of 0 5-0 6 units over a relatively short time

Experiment number	C_{PP}/mM	S_0	C_{EHDP} / µM	pH_p	t_p / h
17	0 09	6 7	0	6 7	1 3
18	0 09	6 4	10	6 7	9
19	0 18	8 6	0	6 8	1 4
20	0 18	8 2	10	6 8	5 7
21	0 36	11 2	0	7 0	1 8
22	0 36	10 6	10	6 9	4 0

Table 5 Spontaneous precipitation of calcium pyrophosphate, at pH ~ 7, with and without the addition of 10 µM EHDP

From table 5 it is seen that the induction time, t_p, at which precipitation occurs from a solution supersaturated with calcium pyrophosphate, is prolonged significantly if EHDP is added For example, the induction time is increased from 1 3 hours to 9 hours when EHDP is added in a concentration of 10 µM (compare experiment number 17 and 18)

Experiment number	C_{PP}/mM	S_0	C_{EHDP} / µM	pH_p	t_p / h
23	0 09	6 7	0	7 4	3
24	0 09	6 4	10	7 0	19
25	0 18	8 6	0	7 5	2 8
26	0 18	8 2	10	7 0	22
27	0 36	11 2	0	7 8	4
28	0 36	10 6	10	7 4	8

Table 6 Spontaneous precipitation of calcium pyrophosphate, at higher pH-values (as compared with table 5), with and without the addition of 10 µM EHDP

From table 6 it is seen that the induction times, t_p, are longer at the higher pH-values (both with and without the addition of 10 µM EHDP) However, it is noted that the

induction time for the onset of spontaneous precipitation is significantly increased upon the addition of 10 µM EHDP

Table 7 below serves to illustrate the spontaneous precipitation of calcium pyrophosphate, CPP, from solutions supersaturated with respect to CPP, but without the addition of crystals In the present example, pH is kept constant at pH 7 0 by the titration with 2 0 mM KOH S_0 is the supersaturation with respect to m-CPPTβ and C_{PP} is the pyrophosphate concentration, $C_{Ca,0} = 2\ C_{PP,0}$ The induction time, t_p, denotes the time, after mixing EHDP and the solution supersaturated with CPP, at which the onset of precipitation is observed, i e as a clear decrease in the pH-value

Experiment number	C_{PP}/mM	S_0	C_{EHDP} / µM	t_p / h
29	0 085	3 9	0	0 5
30	0 085	3 7	10	8
31	0 17	6 2	0	8
32	0 17	6 1	10	2-5 d
33	0 32	9 3	0	7
34	0 32	9	10	15

Table 7 Spontaneous precipitation of solutions supersaturated with respect to calcium pyrophosphate, CPP, with and without the addition of 10 µM EHDP, temperature is 37 0 ± 0 1 °C

From table 7 it is seen that the induction time, at which spontaneous precipitation occurs, t_p, is longer when 10 µM EHDP is added to the solution The largest effect is found for C_{PP} = 0 17 mM, i e the onset of precipitation is observed only on the course of two to five days

Example III

Tables 8a and 8b below serve to illustrate the dissolution of acicular t-CPPD (table 8a) and columnar-CPPD (table 8b) when 10 mg/L of the respective crystal variants are added to an unsaturated solution with $C_{Ca,0} = 2C_{PP,0}$ = 0 07 mM, both with and without the addition of EHDP, pH is kept constant at 5 0 by the titration of 2 0 mM HNO_3

Experiment number	C_{EHDP} / µM	t_f / h	V_H / mL	C_{Ca} / mM
35	0	1 0	11	0 10
36	0	1 3	13	0 10
37	0	1 0	14 5	0 106
38	1	1 0	14 5	0 107
39	10	1 0	14 4	0 109
40	100	1 1	13 1	0 106

Table 8a Dissolution of acicular t-CPPD, when 10 mg/L acicular t-CPPD is added to an <u>unsaturated</u> solution ($C_{Ca,0}$ = 2$C_{PP,0}$ = 0 07 mM), both with and without the addition of EHDP pH is kept constant at 5 0 by the titration of 2 0 mM HNO_3 The supersaturation, S_0, is 0 50, and the temperature is 37 0 ± 0 1 °C

Experiment number	C_{EHDP} / µM	t_f / h	V_H / mL	C_{Ca} / mM
41	0	1	3 3	0 08
42	10	1	2 3	0 08

Table 8b Dissolution of columnar t-CPPD, when 10 mg/L columnar t-CPPD is added to <u>unsaturated</u> solutions ($C_{Ca,0}$ = 2$C_{PP,0}$ = 0 07 mM), both with and without the addition of EHDP pH is kept constant at 5 0 by the titration of 2 0 mM HNO_3 The supersaturation, S_0, is 0 50, and the temperature is 37 0 ± 0 1 °C

From Tables 8a and 8b it is seen that the addition of EHDP, in concentrations of up to 10 µM, has no significant effect on the dissolution rate of acicular or columnar t-CPPD

Tables 9a, 9b and 9c below serve to illustrate the dissolution of 10 mg/L acicular t-CPPD (Table 9a), columnar-CPPD (Table 9b) and monoclinic-CPPT (Table 9c) when added to water, pH kept constant at 7 0 by titration with 2 0 mM HNO_3

Experiment number	C_{EHDP} / µM	t_r / h	V_H / mL	C_{Ca} / mM
43	0	2	1,4	0,005±1
44	10	3	1,4	0,003±1
45	100	3 5	0*	0 008±1

Table 9a Dissolution of acicular t-CPPD when added to water, pH = 7 0, S_0 = 0 *Less acid is required because formation of soluble complexes between Ca^{2+} and EHDP release H^+

Experiment number	C_{EHDP} / µM	t_r / h	V_H / mL	C_{Ca} / mM
46	0	4	4 2	0 018
47	10	4	3 3	0 016
48	100	4	0*	0 020

Table 9b Dissolution of m-CPPD, when added to water, pH = 7 0, S_0 = 0 *Less acid is required because formation of soluble complexes between Ca^{2+} and EHDP release H^+

Experiment number	C_{EHDP}/µM	t_r/h	V_H/mL	C_{Ca}/mM
49	0	4	6 8	0 028
50	10	4	6 1	0 028
51	100	4	≈2*	0 035

Table 9c Dissolution of m-CPPT, pH = 7 0, S_0 = 0 *Less acid is required because formation of soluble complexes between Ca^{2+} and EHDP release H^+

The solubilities of t-CPPD, m-CPPD and m-CPPT in water at pH = 7 0 are 0 009 mM, 0 018 mM and 0 03 mM Ca^{2+}, respectively In Tables 9a, 9b and 9c it is seen that no significant effect of EHDP was observed, i e the dissolution of the added crystals is substantially the same irrespective the addition of EHDP

Example IV

Use of EHDP in the context of calcium pyrophosphate deposition disease

The following example serves to illustrate the use of EHDP for the preparation of a medicament for the treatment of calcium pyrophosphate deposition disease in a mammal It will be appreciated that the present example is not intended in any way to otherwise limit the scope of the invention

The required dosage of the bisphosphonic acid derivative will vary with the particular condition to be treated, the severity of the of the condition, the duration of the treatment and the specific bisphosphonic acid derivative employed However, single oral dosages of the bisphosphonic acid derivative, such as the salt of ethane-1-hydroxy-1,1-bisphosphonic acid, can range from 0 1 to 500 mg per kilogram of body weight, preferably from 0 5 to 250 mg per kilogram of body weight such as from 1 0 to 50 mg per kilogram of body weight Said oral dosages may be administered preferably up to two times daily, such as up to three times daily, preferably such as up to four times daily Dosages greater than, e g , 500 mg per kilogram of body weight may produce toxic symptoms and should be avoided

For purposes of oral administration, the active compound EHDP may be formulated in the form of capsules, tablets or granules, preferably prepared in unit dosage form together with a pharmaceutically acceptable carrier Preferably, the pharmaceutically acceptable carrier comprises from 0 1 to 95 percent by weight of the total composition, such as from 0 1 to 98 percent by weight of the total composition

Without being limited hereto, the active compound EHDP may also be administered parentally in aqueous solution to the subject by subcutaneous, intradermal, intramuscular or intravenous injection Preferably, when administered parentally, the dosage may range from 0 05 to 15 mg per kilogram of body weight or such as from 0 5 to 10 mg per kilogram of body weight

Example V

Use of EHDP in the context of secondary caries

The following example serves to illustrate the physical impact (binding strength) when adding EHDP to dental enamel imbedded in a plastic matrix, and an appropriate means for applying said compound Hence, the example serves to illustrate the binding strength between dental enamel and a plastic filling material, when said dental enamel is treated with EHDP

Preliminary experiments for determination of the binding strength between dental enamel treated or not treated with bisphosphonate and plastic dental filling material

Two comparable pieces of enamel were imbedded in a plastic matrix

1 Both pieces were polished so that a flat part of dental enamel was exposed to air
2 Both pieces of enamel were treated with phosphoric acid, as is the normal practice when using plastic dental filling material
3 Both pieces were rinsed under tap water

4 One of the two pieces was treated with a few drops of 0 1 mol/L of EHDP, neutral pH, for a few seconds This piece was then rinsed with tap water

5 A form with a small cylindrical hole was placed on a specimen

6 The hole was filled with a dental plastic filling material, that does not bind to the form, but which binds to the dental enamel

7 The dental plastic filling material was polymerised by treatment with ultraviolet light, with an lamp used to polymerise this type of pastic filling in normal dental clinics

8 After polymerisation the form was removed, leaving a small cylinder of the plastic filling material on the enamel surface of the specimen

9 The other specimen was treated similarly

10 Both specimens were soaked in water for some time

11 The enamel and the plastic filling cylinder were pulled apart using an apparatus which can measure the force required to separate the two materials

12 For both specimens the binding between enamel and plastic material was broken cleanly

13 The force per unit area required for the specimen not treated with EHDP was 16 5 MPa The force per unit area required for the specimen treated with EHDP was 18 7 MPa

The result above indicates that the binding of the filling material to the tooth enamel is not weakened by the administration of EHDP to the tooth enamel

It will be appreciated that the above example could well be expanded so as to include the measurement of other parameters such as, e g , surface roughness and adhesion It is further anticipated that the chemical stability is able to be measured over time Hence, it is anticipated that the chemical stability between the dental enamel and the plastic material can be monitored over time, both with and without the addition of EHDP to the dental enamel For example, monitoring the increase or decrease of the pH in the specific environment, wherein the two components are placed, will enable the person skilled in the art to elucidate the effect of the addition of EHDP onto the dental enamel

According to the present invention, EHDP is to be applied, in an aqueous solution, onto a tooth subject to primary caries It is anticipated, that the solution is applied onto the enamel *after* the tooth has been treated for the primary caries, according to any conventional method known to the person skilled in the art, but *prior* to the filling of the primary caries with a filling material, such as amalgam or plastic

The required dosage in the present context is such that the concentration of EHDP in the aqueous solution is in the range of from 0 5 to 50 μM, preferably such as from 1 to 10 μM The amount administered to the caries should be in the range of from 0 5 to 5 droplets,

e g , such as from 0 0025 ml to 0 25 ml The amount of droplets is determined by the size or extent of the caries, the requirement is that the entire surface of the caries is substantially covered by a thin layer of the aqueous solution

CLAIMS

1 Use of an alkyl-1,1-bisphosphonic acid derivative of the formula I

$$R^1\text{-}C(PO_3H_2)_2\text{-}R^2 \qquad (I)$$

wherein R^1 is selected from hydrogen and $C_{1\text{-}6}$-alkyl, and R^2 is selected from hydroxy, amino, $-CH_2COOH$, $-CH_2PO_3H_2$ and $-CH_2CH_2PO_3H_2$,

for the preparation of a medicament for the treatment of calcium pyrophosphate deposition disease (CPDD) in a mammal

2 The use according to claim 1, wherein R^1 is $C_{1\text{-}6}$-alkyl and R^2 is hydroxy

3 The use according to claim 2, wherein R^1 is methyl

4 The use according to claim 3, wherein the alkyl-1,1-bisphosphonic acid derivative is ethane-1-hydroxy-1,1-bisphosphonic acid (EHDP)

5 The use according to claim 1, wherein the alkyl-1,1-bisphosphonic acid derivative is methanehydroxybisphosphonic acid

6 The use according to claim 1, wherein the alkyl-1,1-bisphosphonic acid derivative is ethane-1-amino-1,1-bisphosphonic acid

7 The use according to any of the preceding claims, wherein the CPDD is confined to the hyaline cartilage, the fibrocartilage in the meniscus of the knee, the annulus fibrosus of the intervertebral disc, the synovial fluid, or the synovium and tendon insertions

8 The use according to claim 7, wherein the CPDD is confined to the synovial fluid

9 The use according to any of claims 1-6, wherein the CPDD is confined to the articular cartilage

10 Use of an alkyl-1,1-bisphosphonic acid derivative of the formula I

$$R^1\text{-}C(PO_3H_2)_2\text{-}R^2 \qquad (I)$$

wherein R^1 is selected from hydrogen and $C_{1\text{-}6}$-alkyl, and R^2 is selected from hydroxy, amino, $-CH_2COOH$, $-CH_2PO_3H_2$ and $-CH_2CH_2PO_3H_2$,
for the manufacture of a medicament for the prevention or treatment of secondary caries

11 The use according to claim 10, wherein R^1 is $C_{1\text{-}6}$-alkyl and R^2 is hydroxy

12 The use according to claim 11, wherein R^1 is methyl

13 The use according to claim 12, wherein the alkyl-1,1-bisphosphonic acid derivative is ethane-1-hydroxy-1,1-bisphosphonic acid

14 The use according to claim 10, wherein the alkyl-1,1-bisphosphonic acid derivative is methanehydroxybisphosphonic acid

15 The use according to claim 10, wherein the alkyl-1,1-bisphosphonic acid derivative is ethane-1-amino-1,1-bisphosphonic acid

16 The use according to claim 10, wherein the secondary caries is confined to the interface between the natural dental material (enamel, dentine, cementum and root material) and the filling material

17 The use according to claim 16, wherein the filling material is amalgam or plastic